within the recombinant yeast proteasome lid complex that was previously not amenable to labeling by MBP fusion. This technique is extremely versatile and precise; it can be readily expanded to other organisms and to other labels, and will be useful for a wide range of structural analyses.

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Fast Shape-Based Global and Local Electron Density Map Search

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The Electron Microscopy DataBank (EMDB) is growing rapidly, accumulating biological structural data obtained cryo-electron microscopy (cryo-EM). Cryo-EM is an emerging technique for determining large biomolecular complexes and subcellular structures. Together with the Protein Data Bank (PDB), EMDB is becoming a fundamental resource of the tertiary structures of biological macromolecules. To take full advantage of this indispensable resource, the ability to search the database by structural similarity is essential. However, unlike high-resolution structures stored in PDB, methods for comparing low-resolution EM density maps are not well established. Here, we developed a novel computational method for efficiently searching EM maps. The method uses a compact fingerprint representation of EM maps based on the 3D Zernike descriptor, which is a mathematical series expansion for representing isosurface shape of EM maps. The method was implemented in a web server, named EM-SURFER (http://kiharalab.org/em-surfer/), which allows users to search against the entire EMDB with over 2400 entries in a few seconds. By combing with map segmentation, the method can also identify corresponding local regions in EM maps. Examples of search results from different types of query structures are discussed. The unique capability of EM-SURFER to detect 3D shape similarity of low-resolution EM maps should prove invaluable in structural biology.

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Cyclophilin a Stabilizes the Mature HIV-1 Capsid through a Novel Non-Canonical Binding Site

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Host cell factor cyclophilin A (CypA) plays an important role in modulating HIV-1 capsid function. Several other host cell proteins, including TRIMCyp and NUP358, contain a CypA domain that interacts directly with viral capsid. The binding of the host protein CypA to the viral capsid is important for infection, yet the structural effects of this binding have not been identified. We determined the cryoEM structure of CypA in complex with an HIV-1 capsid assembly at 8 Å resolution. The density map displays a non-random, selective binding of CypA along the most curved helical direction, forming a bridge directly above the CA CTD-CTD dimer interface cross the adjacent CA hexamers. CryoEM structure-based modeling and large scale all-atoms molecular dynamics simulations reveal unexpectedly that one CypA molecule simultaneously interacts with two CA molecules through a non-canonical novel interface. The individual residuals critical for the interactions were further identified by solution and solid state NMR and confirmed by mutagenesis studies. Our combined cryoEM, computational and NMR studies provide mechanistic insights into the functional role of CypA in modulating capsid uncoating and viral infectivity, and our structure further highlights the novel CypA and CA interface as an attractive therapeutic target for pharmacological intervention.

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Residue Specific Radiation Damage of Protein Structures using High-Resolution Cryo-Electron Microscopy

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High-resolution structures of proteins and protein complexes are currently determined using either X-ray crystallography, NMR spectroscopy, or now also cryo-electron microscopy (cryo-EM). The highest resolutions achieved by cryo-EM have been typically restricted to large, well-ordered entities such as helical or icosahedral assemblies or 2D crystals. However, we show that emerging methods in single-particle cryo-EM now allow structure determination at near-atomic resolution, even for much smaller protein complexes with low symmetry. We solved the structure of the ~465-kDa Escherichia coli β-galactosidase at ~3.2-Å resolution using single-particle cryo-EM. At this resolution, the majority of the side-chains, the N-termini, and the geometry of the active sites, including a catalytic Mg2+-ion, can be clearly discerned in the density map. Inspection of the map reveals that while densities for residues with positively charged and neutral side-chains are well resolved, systematically weaker densities are observed for residues with negatively charged side-chains. The negatively charged glutamate and aspartate show on average 30% less density than the similarly sized neutral glutamine and asparagine. This observation is independent of the exposure of these residues to solvent. Analysis of other high-resolution cryo-EM structures reveals similar weaker densities for these types of residues. Radiation damage in X-ray crystallography has been linked to decarboxylation of glutamate and aspartate residues, breakage of disulfide bonds, loss of hydroxyl-groups from tyrosine and methylthio-group of methionine. We now show that negatively charged residues exhibit more pronounced effects of radiation damage in structures solved by cryo-EM. We determined that the degree of damage is dose dependent by comparison of density maps obtained using electron doses ranging from 10-30 electrons/Å2. In summary, we establish the feasibility of determining structures at near-atomic resolution and provide a measure of the effects of radiation damage in high-resolution cryo-electron microscopy.

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3D Ultrastructural Investigation of Entire Pancreatic Islets of Langerhans by Serial Block Face Scanning Electron Microscopy

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Serial block-face scanning electron microscopy (SBF-SEM) provides three-dimensional ultrastructure of biological tissues at the nanometer scale (Denk and Horstmann, 2004). Previous studies have applied this technique to visualize the architecture of pancreatic islets of Langerhans in wild-type mice. Initial results indicate that an average beta cell has almost twice the cellular volume of an average alpha cell and four times the mitochondrial volume, whereas the nuclear volumes in both cell types are approximately equal. Comparisons of insulin-secreting beta granules and glucagon-secreting alpha granules show that the beta granules have more pronounced halos and diameters twice that of the alpha granules. In addition, three-dimensional rendering of islet blood vessels reveals that all secretory cells in an islet are in contact with the pericapillary space with an average contact area of 9% +/− 5% of the plasma membrane's surface. We are currently applying SBF-SEM to determine quantitatively how islet morphology differs between genotypes in wild type, non-obese diabetic (NOD) and IA-2/IA-2 beta double-knockout mice. Such quantitative analysis of ultrastructural differences between controls and diabetes-related animal models could help further understanding of disease. The research was supported by the intramural programs of NIBIB and NIDCR.

Denk, W., & Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS biology, 2(11), e329.

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Superresolution Fluorescence Microscopy within a Scanning Electron Microscope

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Correlative optical and electron microscopy has the potential to provide detail on the organization of biological static structures at multiple length scales, but conflicting technical requirements on experimental design, molecular identification, and sample preparation present major obstacles. While fluorescence microscopy is usually impossible in an electron microscope, we present nondestructive fluorescence imaging of biological samples in an SEM. Our novel chamber allows a scanned electron beam to indirectly excite fluorescence with nanometer-scale resolution while simultaneously protecting the sample